

These observations, perhaps in conjunction with the known physiological role of IL-6/sIL-6R in leukocyte and T-cell recruitment, suggest that Ad vectors encoding HIL-6 may have utility as therapeutic agents for treating cancers of various types.

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Efficient gene delivery into human primary glioma cells by fiber retargeted adenoviral vectors: implications for *in vivo* gene delivery into malignant gliomas

X Fan¹, I Sloots¹, S Strömbäck¹, L G Salford², B Widgren¹

¹Lund University, Department of Tumor Immunology, Lund, Sweden; ²Lund University, Neurosurgery, Lund, Sweden

Contact e-mail: Xiaolong.Fan@molmed.lu.se

Malignant glioma is so far an incurable disease. Adenoviral vector mediated gene delivery can be a useful tool in the development of novel treatments. However, the glioma cells from many patients can not be efficiently transduced by adenovirus serotype 5 (Ad5) based vectors due to a paucity of the coxsackie and adenovirus receptor (CAR), which mediates the adenoviral fiber attachment to the host cells. In this study, we hypothesized that the CAR-dependent transduction can be overcome by modifying the fiber gene in the adenoviral vectors. Glioma cell lines were generated from the surgical specimens. Thirty to 66% of the cells from patients GF-34, GA-49 and AMN-39 show high levels of CAR expression, whereas only 3 to 11% of the cells from patients DZ-48, KN-35 and BP-34 show measurable levels of CAR expression. The transduction efficiency was compared between the Ad5-GFP and Ad5F35-GFP vectors, both encode the GFP gene driven by the PGK-1 promoter. The latter carries an engineered fiber gene with the specificity of Ad35 fiber. Seven percent of the DZ-48 cells were GFP+ after a 48-hr transduction with the Ad5-GFP vector at an MOI of 100. In contrast, 87% of the DZ-48 cells were GFP+ upon Ad5F35-GFP transduction. In cells from AMN-39 and GF-34 patients, approximately 75% and 95% of the cells were GFP+ after transduction with the Ad5-GFP and Ad5F35-GFP vector, respectively. Importantly, the uptake of the Ad5F35-GFP vector into glioma cells was to large extent complete in the first 1-hr of transduction; equal transduction efficiency into the DZ-48 cells by the Ad5-GFP vector was not achieved even by using longer transduction period and a higher MOI. In conclusion, the Ad5F35-GFP vector drastically improved the gene transfer efficiency into the primary glioma cells in a CAR-independent manner. Our findings facilitate the future development of novel treatments against gliomas.

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Adenovirus-mediated transfer of angiostatic genes in a human *in vitro* angiogenesis model

K Rittner, L Laruelle, G Cauet, M Lusky

TRANSGENE S.A., Adenovirology, Strasbourg, France

Contact e-mail: rittner@transgene.fr

Background: Intense interest is currently focused on angiogenesis, the multistep process whereby new blood vessels develop from pre-existing vasculature. This derives from the key role of angiogenesis in numerous physiological and pathological processes including tumor growth and metastasis development.

Objective: Our goal was to identify a relevant *in vitro* assay to test angiostatic genes encoded by adenoviral vectors.

Methods: We have chosen a commercially available human angiogenesis assay, based on the co-culture of human primary fibroblasts

and human umbilical vein endothelial cells (HUVEC). The endothelial cells initially form small islands within the culture matrix. They subsequently begin to proliferate and then enter a migratory phase during which they move through the extracellular matrix provided by the fibroblasts to form threadlike tubule structures. The morphology of the tubules resembles capillary formation *in vivo*. Inhibitory as well as stimulatory effects can be quantified by measuring the total tubule length as well as the branch points which give dose response curves when plotted against the concentration of test compounds like small molecules.

Results: We have validated this test for the use of adenovirus-mediated gene transfer. After infection with an E1-deleted "empty" adenoviral vector (AdE1^o), tubule length decreased and the number of remaining endothelial islands increased compared to untreated controls. Since it is preferentially the endothelial cells which are infected as shown using a beta-galactosidase-encoding adenoviral vector, this effect might be due to interference of viral infection with migration and differentiation of the endothelial cells. In contrast, infection with AdE1^o vectors encoding the soluble forms of the human and murine VEGF receptor Flt1 resulted in complete inhibition of tubule formation and in an even more increased number of endothelial islands.

Conclusion: The observed differences between AdE1^o and soluble VEGF receptor-encoding vectors are significant. This allows to analyze the reduction / inhibition of VEGF-dependent tubule formation in this human *in vitro* angiogenesis assay delivering potentially angiostatic genes with adenoviral vectors.

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***In vitro* reduction of VEGF secretion from the human glioblastoma cell line U87MG through an anti-VEGF ribozyme. The inhibition of angiogenic potential as a gene therapy approach for brain tumors**

S A Ciafre, F Niola, F Wannenes, M G Farace

University of Rome Tor Vergata, Experimental Medicine and Biochemical Sciences, Rome, Italy

Contact e-mail: ciafre@uniroma2.it

Background: glioblastoma multiforme is one of the most highly vascularized solid neoplasms, with the amount of neovasculture closely correlated with the degree of malignancy, characterised by an up-regulation of vascular endothelial growth factor (VEGF) expression. Objective: the aim of our research is to design an efficient molecular tool that strongly down-regulates expression and secretion of VEGF from a human glioblastoma cell line, in order to reduce its proangiogenic and tumorigenic potential. To reach this goal, we have designed an anti-VEGF ribozyme and we have cloned it into a molecular milieu which is expected to enhance and support the anti-VEGF cleaving activity of our ribozyme.

Methods and Results: the ribozyme was cloned into into a short region of Adenovirus type 2, VAI, owning several features making it an optimal carrier for efficient and specific delivery of ribozymes into the cytoplasm: VAI is transcribed by the RNA Pol III, driving high levels of expression, its secondary and tertiary structures confer high stability to the transcribed molecules, and VAI is specifically sorted into the cytoplasm, where the activity of the ribozyme is required. We have tested the efficacy of the plasmid harbouring the anti-VEGF ribozyme-VAI "hybrid" molecules, by transfecting a human glioma cell line, U87MG. In a transient transfection assay, VEGF-ELISA measurement has shown a significant reduction (up to 55% decrease) of VEGF secretion from transfected cells, in a strict temporal correlation with the expression of the ribozyme (measured by RT-PCR and Northern blot). Though, as it is predictable that an efficient